(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
29 April 2004 (29.04.2004)

PCT

(10) International Publication Number
WO 2004/036898 A3

(51) International Patent Classification[7]: G02B 21/00, H04N 5/225

(21) International Application Number: PCT/GB2003/004486

(22) International Filing Date: 15 October 2003 (15.10.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0224067.9 16 October 2002 (16.10.2002) GB

(71) Applicant *(for all designated States except US)*: **PERKINELMER UK LIMITED** [GB/GB]; 204 Cambridge Science Park, Milton Road, Cambridge CB4 OGZ (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **BOBANOVIC, Fedja** [GB/GB]; 31 The Paddocks, Cambridge CB1 3HG (GB). **PHILLIPS, John** [GB/GB]; 40 Roseford Road, Cambridge CB4 2HD (GB). **LADHA, Shab** [GB/GB]; 18 The Rowans, Milton, Cambridge CB6 6YU (GB). **COURTNEY, Patrick** [GB/GB]; 81 Riverside, Cambridge CB5 8HN (GB).

(74) Agent: **KEITH W NASH & CO.**; 90-92 Regent Street, Cambridge CB2 1DP (GB).

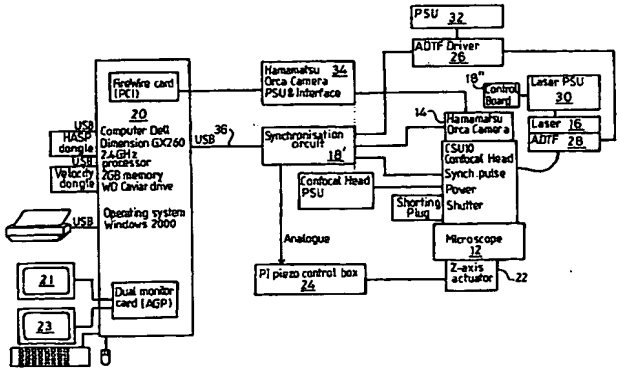
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

[Continued on next page]

(54) Title: IMPROVEMENTS IN AND RELATING TO IMAGING



(57) Abstract: A method of imaging light from a specimen in which excitation light passes to the specimen via a scanning system and light emitted by luminescence of the specimen passes in another direction via the scanning system to an image capture device having a sensor having discrete spatially distinct light sensitive regions. The scanning system is operated so as to scan the whole of an area of interest of the specimen, and the excitation light and/or the image capture device are controlled so that light emitted from the specimen is only incident on the image capture device sensor for a specific time period equal to that required for scanning the whole of the area of interest n times (where n is a whole number equal to or greater than 1). The scanning system is a confocal system, and in one example comprises a rotating Nipkow disc scanner and the pattern of openings in the disc is such that rotation of the disc through A° results in scanning the whole of the area of interest and the said specific time period is selected to correspond to nA° of disc rotation. Apparatus for performing the method to produce a video signal for creating an image in a display device or for processing and analysis by a computer, includes control means adapted to control the excitation light and/or the image capture device so that light from the specimen is incident on the image capture device for the specific period of time, and the image capture device is a CCD camera.

WO 2004/036898 A3

(88) Date of publication of the international search report:
17 June 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

Internat Application No
PCT/GB 03/04486

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G02B21/00 H04N5/225

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G02B H04N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, PAJ, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99/63381 A (MUEHLIG PETER ;CZARNETZKI NORBERT (DE); ZEISS CARL JENA GMBH (DE)) 9 December 1999 (1999-12-09) page 7, line 27-37 page 8 page 9, paragraph 1 - paragraph 30 figures 1A,1B ---	1,97
Y	US 2002/097490 A1 (EDA YUKIO ET AL) 25 July 2002 (2002-07-25) paragraph '0164! - paragraph '0181! figures 13-15E ---	1,97
Y	US 4 566 029 A (JOHNSON CYDNEY A) 21 January 1986 (1986-01-21) abstract; figures ---	1,97
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 April 2004	27/04/2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Ward, S

Form PCT/ISA/210 (second sheet) (January 2004)

INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/GB 03/04486

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DE 196 27 568 A (ZEISS CARL JENA GMBH) 15 January 1998 (1998-01-15) abstract; figures	1,97
A	TANAAMI T ET AL: "HIGH-SPEED 1-FRAME/MS SCANNING CONFOCAL MICROSCOPE WITH A MICROLENS AND NIPKOW DISKS" APPLIED OPTICS, OPTICAL SOCIETY OF AMERICA,WASHINGTON, US, vol. 41, no. 22, 1 August 2002 (2002-08-01), pages 4704-4708, XP001131422 ISSN: 0003-6935 abstract; figure 1	1,97
A	US 4 910 606 A (KAJI TOSHIO ET AL) 20 March 1990 (1990-03-20) abstract; figures	1,97
A	PATENT ABSTRACTS OF JAPAN vol. 015, no. 018 (E-1023), 16 January 1991 (1991-01-16) -& JP 02 266674 A (KYOCERA CORP), 31 October 1990 (1990-10-31) abstract; figures	1,97
A	US 5 969 846 A (KISHI YOSUKE) 19 October 1999 (1999-10-19) abstract; figures	1,97
A	KAWAMURA S ET AL: "CONFOCAL LASER MICROSCOPE SCANNER AND CCD CAMERA" YOKOGAWA TECHNICAL REPORT ENGLISH EDITION , 'Online! no. 33, 2002, XP002277034 Retrieved from the Internet: <URL:http://www.yokogawa.com/rd/pdf/TR/rd-tr-r00033-005.pdf> 'retrieved on 2004-04-15! cited in the application the whole document	1,97

Form PCT/ISA/210 (continuation of second sheet) (January 2004)

INTERNATIONAL SEARCH REPORT

Inte nal application No.
PCT/GB 03/04486

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 2-96,98-172
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 2-96,98-172

The present application has 172 claims on file. Furthermore, the search for the subject-matter of independent claims 1 and 97 has revealed that these claims do not appear to be novel in the light of WO9963381 A, and that as a result 70 new independent claims emerge. It is therefore difficult, if not impossible, to determine the matter for which protection is sought, the present application fails to comply with the clarity and conciseness requirements of Article 6 PCT (see also Rule 6.1(a) PCT) to such an extent that a meaningful search is impossible. Consequently, the search has been carried out for those parts of the application which do appear to be clear (and concise), namely claims 1 and 97 (particularly, but not exclusively having regard to confocal microscopy) and the general concept of periodic prevention of light from being incident on image capture devices.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Internal application No
PCT/GB 03/04486

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9963381 A	09-12-1999	DE 19824460 A1	02-12-1999
		WO 9963381 A1	09-12-1999
		JP 2002517774 T	18-06-2002
		US 6545265 B1	08-04-2003
US 2002097490 A1	25-07-2002	JP 2000275534 A	06-10-2000
		JP 2000275530 A	06-10-2000
		JP 2000275540 A	06-10-2000
		JP 2000275542 A	06-10-2000
		DE 10014331 A1	05-10-2000
		US 6426835 B1	30-07-2002
US 4566029 A	21-01-1986	NONE	
DE 19627568 A	15-01-1998	DE 19627568 A1	15-01-1998
US 4910606 A	20-03-1990	JP 4074912 B	27-11-1992
		JP 58116878 A	12-07-1983
		JP 58116879 A	12-07-1983
		JP 1659302 C	21-04-1992
		JP 2030633 B	09-07-1990
		JP 58116880 A	12-07-1983
		JP 1659303 C	21-04-1992
		JP 2030634 B	09-07-1990
		JP 58116881 A	12-07-1983
		JP 1559463 C	16-05-1990
		JP 58116882 A	12-07-1983
		JP 63004998 B	01-02-1988
		JP 58116883 A	12-07-1983
		US 5760830 A	02-06-1998
		US 5309247 A	03-05-1994
		US 4599657 A	08-07-1986
		US 5010418 A	23-04-1991
		US 4763204 A	09-08-1988
JP 02266674 A	31-10-1990	NONE	
US 5969846 A	19-10-1999	JP 10161032 A	19-06-1998

CLAIMS

1. A method of imaging light from a specimen in which excitation light passes to the specimen via a scanning system and light emitted by luminescence of the specimen passes in another direction via the scanning system to an image capture device having a sensor having discrete spatially distinct light sensitive regions, and the scanning system is operated so as to scan the whole of an area of interest of the specimen, wherein the excitation light and/or the image capture device are controlled so that light emitted from the specimen is only incident on the image capture device sensor for a specific time period equal to that required for scanning the whole of the area of interest n times (where n is a whole number equal to or greater than 1).

2. A method as claimed in claim 1 wherein the area of interest is the whole of the viewable area of the specimen.

3. A method as claimed in claim 1 wherein the area of interest is a part of the visible area of the specimen, defined by a window of variable size and location created by the scanning system.

4. A method as claimed in any of claims 1 to 3 wherein the scanning system is a confocal system

5. A method as claimed in any of claims 1 to 4 wherein the scanning system comprises a rotating Nipkow disc scanner in which excitation light passes through openings in the disc in one direction and light emitted by luminescence of the specimen passes through the openings in the opposite direction to form an image at the image capture device.

6. A method as claimed in claim 5 wherein the pattern of openings is such that rotation of the disc through A° results in scanning the whole of the area of interest and the said

specific time period is selected to correspond to nA^{o} of rotation of the disc (where n is a whole number equal to or greater than one).

7. A method as claimed in claim 5 or 6 wherein the Nipkow disc scanner includes two discs.

8. A method as claimed in claim 7 wherein one of the two discs contains openings and the other microlenses.

9. A method as claimed in any of claims 1 to 4 wherein the scanning is effected in a series of lines in the form of a raster scan.

10. A method as claimed in claim 9 wherein the scanning is effected using a pinhole.

11. A method as claimed in claim 9 wherein the scanning is effected using a plurality of pinholes in a fixed pattern.

12. A method as claimed in any of claims 1 to 4 wherein the scanning is effected using a scanning slit scanner.

13. A method as claimed in any of claims 1 to 4 wherein confocality is achieved by a 2 photon process which limits the focal plane of activation.

14. A method as claimed in any of claims 1 to 4 wherein confocality is obtained by a time delay multiplexed process.

15. A method as claimed in any of claims 1 to 4 wherein an array of independently controllable mirrors is used to effect the scanning.

16. A method as claimed in any of claims 1 to 4 wherein the scanning is effected using a random or quasi random pattern of transmissive and reflective elements.

17. A method of as claimed in any of claims 1 to 16 wherein excitation light is prevented from reaching the specimen except for each of a succession of separated specific periods of time and the image capture device is read out at the end of each specific period of time or at the end of a plurality of consecutive specific periods of time.

18. A method as claimed in claim 17 wherein the image capture device is only receptive of light emitted by the specimen during each specific period of time, and after the image signals are read out from the capture device, the capture device is reset.

19. A method as claimed in any of claims 1 to 18 in which the light source is switched on and off to control the duration of the exposure.

20. A method as claimed in any of claims 1 to 18 wherein shutter means is provided to interrupt light from the excitation source except for when the specimen is to be scanned.

21. A method as claimed in claim 20 wherein an intrinsic (electronic) shutter is used to interrupt the excitation light.

22. A method as claimed in any of claims 1 to 18 wherein a light chopper is used to interrupt the excitation light.

23. A method as claimed in any of claims 1 to 18 wherein an acousto-optic light interrupting element is used.

24. A method as claimed in any of claims 20 to 23 wherein the excitation light source operates to produce light for periods of time corresponding to but of linger duration than the said specific periods of time.

25. A method as claimed in any of claims 20 to 23 wherein the excitation light source is operated continuously.

26. A method as claimed in any of claims 1 to 25 wherein shutter means is provided for both excitation source and image capture device.

27. A method as claimed in claim 26 wherein both shutter means are synchronously operated.

28. A method as claimed in claim 26 wherein electronic pseudo shutter means is employed.

29. A method as claimed in any of claims 1 to 25 wherein shutter means is provided which in use operates to prevent light reaching at least part of the image capture device sensor, except for the said specific periods of time during which the excitation light is incident on the specimen, for the purpose of reducing errors which can arise from light arising from phosphorescence, afterglow, stray reflections or other effects, from reaching the capture device sensor.

30. A method as claimed in claim 29 wherein the shutter means prevents light from reaching all of the image capture device sensor.

31. A method as claimed in any of claims 1 to 30 wherein the specimen is at least in part transparent and a plurality of images are formed by scanning the specimen in a plurality of different spaced apart planes.

32. A method as claimed in claim 31 wherein the different planes are produced by relative movement between the specimen and a scanning device forming part of the scanning system.

33. A method as claimed in claim 32 wherein the specimen movement is achieved by moving a holder containing the specimen.

34. A method as claimed in claim 31 wherein the different planes are produced by movement of at least one part of an optical system forming part of the scanning system

so that light is brought to a focus in the specimen at different spaced apart points, each point therefore defining the position of a focal plane of the scanning system.

35. A method as claimed in claim 34 wherein the different focal planes are obtained by moving an objective lens in the optical system.

36. A method as claimed in any of claims 32 to 35 wherein movement is restricted to periods during which excitation light is not incident on the specimen.

37. A method as claimed in claim 34 wherein movement is restricted to periods during which the image capture device is rendered insensitive to light.

38. A method as claimed in any of claims 32 to 37 wherein the axis of displacement is normal to the or each focal plane of the scanning device.

39. A method as claimed in any of claims 32 to 37 wherein the axis of displacement subtends an angle of other than 90° to the or each focal plane of the scanning device.

40. A method as claimed in any of claims 32 to 39 wherein the movement occurs at the end of each said specific time period or after N successive such time periods (N being an integer equal to or greater than 1).

41. A method as claimed in claim 40 wherein each movement between exposures is the same size.

42. A method as claimed in claim 40 wherein the size of each movement differs from that of a preceding or following movement.

43. A method as claimed in claim 40 wherein each movement is composed of a succession of discrete steps, each of a predetermined equal size.

44. A method as claimed in claim 43 wherein each movement between exposures is made up of M discrete steps (where M is a whole number equal to or greater than 1).

45. A method as claimed in any of claims 32 to 44 wherein the method includes the step of controlling the excitation illumination or the image capture device exposure, or both, by reference to the said movements, such that excitation illumination and/or exposure is only initiated after each movement has been completed.

46. A method as claimed in any of claims 32 to 44 wherein the time between excitation and/or exposures is controlled so as to be at least equal to the time required to complete each movement.

47. A method as claimed in any of claims 32 to 39 wherein the movement is continuous for the purpose of speeding up the scanning of a specimen.

48. A method as claimed in claim 47 wherein the continuous movement during the imaging results in blurring of the image, and the method includes the step of applying deconvolution to re-sharpen the image.

49. A method as claimed in any of claims 32 to 48 wherein the excitation light is composed of light having two or more different wavelength(s).

50. A method as claimed in any of claims 32 to 49 wherein the wavelength of the excitation light is varied between one excitation and/or exposure and the next.

51. A method as claimed in claim 50 wherein the wavelength of the excitation light is varied continuously during a succession of excitations and exposures.

52. A method as claimed in claim 50 wherein the excitation light wavelength is varied between the end of one excitation and/or exposure and the beginning of the next.

53. A method as claimed in claim 50 or 51 wherein the variation of excitation light wavelength is effected whilst imaging the same plane of the specimen.

54. A method as claimed in any of claims 49 to 53 wherein there is no movement within the optical system or of the specimen between each of a succession of two or more excitation and exposure steps making up a sequence of exposures, and the said relative movement is initiated only after each said sequence of exposures has been performed, and the next sequence of such excitation and exposure steps is only initiated after a movement has been completed.

55. A method as claimed in any of claims 32 to 54 wherein the said movement is alternately first in one direction and then in another.

56. A method as claimed in any of claims 50 to 55 wherein wavelength variation is achieved by employing two or more light sources of different peak wavelengths and selectively operating the sources or selectively interrupting light therefrom.

57. A method as claimed in any of claims 49 to 55 wherein two or more light sources having different peak wavelengths are employed, and light is selectively directed from the sources in turn, as required, to the scanning device and specimen.

58. A method as claimed in any of claims 49 to 55 wherein the or each of the light sources used has a complex spectral characteristic.

59. A method as claimed in any of claims 49 to 58 wherein the excitation light is obtained from one or more laser light sources.

60. A method as claimed in claim 59 wherein the or each light source is a directly driven laser diode.

61. A method as claimed in any of claims 49 to 55 wherein a single excitation light source is employed which comprises an acousto optic tuneable filter (AOTF) crystal and the wavelength of the emitted light is varied by altering the frequency controlling signal to the crystal as required.

62. A method as claimed in claim 61 wherein any alteration of the frequency of the controlling signal is effected between exposures.

63. A method as claimed in any of claims 49 to 55 in which the excitation light source comprises a single laser light source, or two or more laser light sources whose outputs are optically combined.

64. A method as claimed in any of claims 1 to 63 wherein the excitation light is pulsed.

65. A method as claimed in any of claims 1 to 64 wherein the excitation light is polarised.

66. A method as claimed in any of claims 1 to 58 wherein a luminescent or incandescent light source is used as the source of excitation light, and an optical filter or a monochromator is employed to control the wavelength of the light emitted by the source.

67. A method as claimed in any of claims 1 to 58 wherein a light emitting (LED) source is used which comprises one or more light-emitting elements.

68. A method as claimed in any of claims 1 to 67 wherein the intensity of the excitation light incident on the specimen is adjusted from one exposure to another.

69. A method as claimed in any of claims 1 to 67 wherein the method involves the step of adjusting the intensity of the incident excitation light between exposures.

70. A method as claimed in claim 68 or 69 wherein the intensity of the incident excitation light is adjusted by interposing neutral density filters, or opening or closing an iris diaphragm in the light path, adjusting the power to the light source, or employing an attenuating element such as an AOTF or LCD shutter, or any combination thereof.

71. A method as claimed in claim 69 or 70 wherein the intensity of the excitation light is adjusted so as to provide a predetermined intensity of illumination at the specimen.

72. A method as claimed in claim 71 wherein the specimen is illuminated by light at different wavelengths and the intensity is adjusted to produce a predetermined level of excitation intensity at the specimen for each wavelength.

73. A method as claimed in claim 71 or 72 wherein the adjustment produces a substantially similar level of intensity at the specimen for each different wavelength.

74. A method as claimed in any of claims 71 to 73 wherein the intensity adjustment is achieved by varying the power to the or each excitation light source.

75. A method as claimed in any of claims 71 to 73 wherein the intensity adjustment is achieved by attenuating excitation light from the or each excitation light source.

76. A method as claimed in claim 74 or 75 wherein the power adjustment or attenuation is adjusted to provide substantially constant intensity luminescence from the specimen irrespective of wavelength.

77. A method as claimed in any of claims 1 to 76 wherein a single control centre produces signals suitable for driving, or controlling power to drives, or to control power to devices to effect movements and rotations required by the different steps of the method.

78. A method as claimed in any of claims 1 to 77 wherein the image capture device is a CCD which provides image data in analogue or digital format.

79. A method as claimed in claim 78 wherein the CCD camera is cooled to increase the S/N ratio of the camera output signals.

80. A method as claimed in any of claims 1 to 77 wherein the image capture device is a CMOS camera, a CID (charge injection device) camera, an intensified or gated camera, a photomultiplier tube array, a photodiode array or an image capture device employing an addressable microcolometer array, or a chemical film camera, or other light sensitive sensor.

81. A method as claimed in any of claims 1 to 80 further comprising the step of forming an image using the light emitted by the specimen which in use is viewed through an eyepiece or is projected by a projection system onto a screen for viewing.

82. A method as claimed in claim 81 wherein the light from the specimen is split by a beam-splitting or beam directing device whereby the light can be split (or divided) between the image capture device and an eyepiece or projection system.

83. A method as claimed in any of claims 1 to 82 wherein a plurality of image capture devices are employed, each of which is supplied with light from the specimen.

84. A method as claimed in claim 83 wherein the or each image capture device is similar to the other image capture device(s).

85. A method as claimed in claim 83 wherein at least one of the image capture devices is of a different type from at least one of the other image capture devices.

86. A method as claimed in any of claims 83 to 85 wherein light from the specimen is split between the plurality of image capture devices.

87. A method as claimed in any of claims 83 to 85 wherein the light from the specimen is directed in different directions so as to be separately received by each of the plurality of image capture devices.

88. A method as claimed in any of claims 1 to 87 wherein image signals from the or each image capture device are supplied to a computer programmed to process and/or analyse the image signals.

89. A method as claimed of claim 88 wherein image signals from the image capture device and/or the computer produce an image in or on a display device or a projected image, for visual inspection.

90. A method as claimed in any of claims 1 to 87 wherein the image capture device signals are stored and subsequently read out for processing and analysis by a computer and/or to produce an image in or on a display device or a projected image, for visual inspection.

91. A method as claimed in any of the preceding claims wherein the excitation light is directed obliquely or axially towards the specimen.

92. A method as claimed in any of the preceding claims in which the excitation illumination of the specimen is dark field illumination, Rheinberg illumination, phase contrast illumination, or Differential Interference Contrast (DIC) illumination.

93. A method as claimed in any of the preceding claims wherein Hoffman Modulation contrast is employed.

94. A method as claimed in any of claims 1 to 91 wherein the excitation light is polarised.

95. A method as claimed in any of claims 1 to 90 wherein the excitation light is in the non-visible part of the spectrum.

96. A method as claimed in claim 95 wherein the excitation light is infrared light.

97. Apparatus by which light emitted from a specimen is imaged by an image capture device to produce a video signal for creating an image in a display device or for processing and analysis, comprising:-

- means for mounting the specimen,

- a light source for producing excitation light,

- a scanning system adapted to direct excitation light in one direction towards, and thereby to scan an area of the specimen and also adapted to convey light emitted from the specimen as a consequence of the excitation light incident thereon, in another direction, which operates in use to scan typically repeatedly an area of interest of the specimen,

- an image capture device having discrete spatially distinct light sensitive regions on which light emitted from the specimen is focussed to form an image after being conveyed through the scanning system in the said other direction, and

- control means adapted to control the excitation light and/or the image capture device so that light from the specimen is incident on the image capture device for a specific time period equal to that required by the scanning system to scan the area of interest n times (where n is a whole number equal to or greater than 1).

98. Apparatus as claimed in claim 97 wherein the area of interest is the whole of the viewable area of the specimen.

99. Apparatus as claimed in claim 97 the scanning system is adapted to generate a window of variable size and location to enable some part of the area of the specimen to be scanned, to restrict the area of interest to part of the viewable area of the specimen.

100. Apparatus as claimed in claim 97, 98 or 99 wherein the scanning system is a confocal system.

101. Apparatus as claimed in any of claims 97 to 100 wherein the scanning system comprises a rotating Nipkow disc scanner in which excitation light passes through openings in the disc in one direction and light emitted by the specimen passes through the openings in the opposite direction to form an image on a sensor in the image capture device, and the pattern of openings is such that rotation of the disc through A° results in scanning the whole of the area of interest, and the said specific time period is selected to correspond to nA° of rotation of the disc (where n is equal to one or a whole number greater than one).

102. Apparatus as claimed in claim 100 wherein the Nipkow disc scanner comprises a 2-disc arrangement.

103. Apparatus as claimed in claim 102 in which one of the discs contains microlenses in its openings.

104. Apparatus as claimed in any of claims 97 to 100 wherein the scanning system incorporates a pinhole and one complete scan using the pinhole corresponds to rotation of a Nipkow disc.

105. Apparatus as claimed in any of claims 97 to 100 wherein the scanning system incorporates a plurality of pinholes arranged in a fixed pattern, and one complete scan using the fixed pattern of pinholes corresponds to rotation of a Nipkow disc.

106. Apparatus as claimed in any of claims 97 to 100 wherein the scanning system incorporates a scanning slit scanner.

107. Apparatus as claimed in any of claims 97 to 100 wherein confocality is achieved by a 2 photon process which includes a 2 photon excitation source and limits the focal plane of activation, and wherein one pulse of the 2 photon excitation source corresponds to rotation of a Nipkow disc.

108. Apparatus as claimed in claim 97 to 100 wherein confocality is obtained by a time delay multiplexed process involving pinholes, wherein one complete scan of the pinholes corresponds to rotation of a Nipkow disc.

109. Apparatus as claimed in any of claims 97 to 100 wherein the scanning system employs an array of independently controllable mirrors and means for adjusting same, which in use synthesise an array of pinholes, and wherein one complete scan of the mirrors to create an image or subimage corresponds to rotation of a Nipkow disc.

110. Apparatus as claimed in any of claims 97 to 100 wherein the scanning system employs a random or quasi random pattern of transmissive and reflective elements which are scanned to produce an image, where one complete scan of the transmissive and reflective elements corresponds to rotation of a Nipkow disc.

111. Apparatus as claimed in any of claims 97 to 110 further comprising means which prevents light from reaching the specimen except from the excitation source, via the scanning system.

112. Apparatus as claimed in claim 111 further comprising means which prevents light from reaching the image capture device except from the specimen, via the scanning system.

113. Apparatus as claimed in any of claims 97 to 112 wherein means is provided by which light from the excitation light source is only incident on the specimen during each said specific time period.

114. Apparatus as claimed in any of claims 97 to 113 wherein the control means also controls the operation of the image capture device whereby it is read out at the end of each said specific time period and is reset prior to the beginning of the next specific time period.

115. Apparatus as claimed in claim 113 wherein the control means switches the excitation light source on and off to control the exposure duration so that the image capture device exposure is in turn governed or controlled by the time for which the excitation light source is active.

116. Apparatus as claimed in claim 113, 114 or 115 further comprising shutter means which in use is operated by signals from the control means to interrupt light from the excitation source except for when the specimen is to be illuminated.

117. Apparatus as claimed in claim 116 wherein the shutter means comprises an acousto-optic element.

118. Apparatus as claimed in any of claims 113 to 116 wherein the light is controlled by an intrinsic shutter.

119. Apparatus as claimed in any of claims 113 to 118 further comprising a light chopper for controlling the passage of excitation light to the specimen.

120. Apparatus as claimed in any of claims 113 to 119 further comprising second shutter means between the scanning system and the image capture device, which second shutter means is operated by signals from the control means so that in use light is prevented from reaching at least part of the image capture device sensor, except for the

specific periods of time during which excitation light is incident on the specimen, for the purpose of reducing errors which could arise from phosphorescence, afterglow, stray reflections or light due to other effects, reaching the capture device.

121. Apparatus as claimed in claim 120 in so far as it is dependent on any of claims 116 to 119 wherein synchronising means is provided whereby in use, the two shutter means are operated synchronously.

122. Apparatus as claimed in any of claims 97 to 121 wherein the scanning system focuses the excitation light in a plane which contains or comprises the area of the specimen to be illuminated by the excitation light.

123. Apparatus as claimed in claim 122 which further includes drive means adapted to move the specimen, the scanning system, or an element of an optical system within the scanning system, along a linear axis (the Z axis) so that in use the position of the plane can adjusted relative to the specimen.

124. Apparatus as claimed in claim 123 wherein the linear axis drive means is also controlled by signals from the control means.

125. Apparatus as claimed in claim 123 or 124 wherein in use the control system operates so as to restrict movement along the linear axis to periods during which light is prevented from reaching the image capture device.

126. Apparatus as claimed in claim 123, 124 or 125 wherein in use the control system only produces movement along the linear axis during periods in which the excitation source light is inhibited or prevented from reaching the specimen.

127. Apparatus as claimed in any of claims 123 to 126 wherein the control means controls the linear axis drive in use so as to effect the linear axis movement at the end

of each said specific time period, or after a succession of N such said specific time periods (N being an integer equal to or greater than 1).

128. Apparatus as claimed in any of claims 123 to 127 wherein the control system is adapted to control the distance moved by the specimen or scanning system or element thereof, along the linear axis.

129. Apparatus as claimed in claim 128 wherein in use the control system controls the linear axis drive means so that each movement of the specimen, or scanning system, or element thereof, produced by the drive means along the linear axis, is of the same size.

130. Apparatus as claimed in any of claims 123 to 127 wherein in use the control system controls the size of each movement of the specimen, or scanning system, or element thereof, along the linear axis, whereby each movement therealong may differ in size from those before and/or following.

131. Apparatus as claimed in any of claims 123 to 130 wherein the control system is adapted in use to control the linear axis drive means to move in discrete steps each of a predetermined equal size, and each linear axis movement of the specimen, or scanning system, or element thereof, is made up of one, or more than one, of the said discrete steps.

132. Apparatus as claimed in any of claims 123 to 131 wherein in use the control means controls the specimen illumination and image capture device exposure, by reference to the linear axis movement such that excitation illumination and image capture device exposure is only initiated after a linear movement has been completed.

133. Apparatus as claimed in any of claims 123 to 131 wherein in use the control means controls the time between exposures so that a sufficient period of time is provided in which the linear axis movement between exposures can be completed.

134. Apparatus as claimed in claim 123 or 124 wherein the linear axis motion of the specimen, or scanning system, or element thereof, is continuous.

135. Apparatus as claimed in claim 134 further comprising means by which deconvolution is applied to re-sharpen the image at the image capture device, or an image produced by signals from the image capture device, which is otherwise blurred due to the said continuous motion.

136. Apparatus as claimed in any of claims 97 to 135 wherein in use the wavelength of the excitation light is required to vary from one exposure to another, and the apparatus comprises two or more excitation light sources each producing excitation light of a different wavelength from the or each other source, and the control means is adapted in use to select the source to provide light of appropriate wavelength for each exposure.

137. Apparatus as claimed in any of claims 97 to 135 wherein in use the wavelength of the excitation light is required to vary from one exposure to another and the apparatus comprises a single source of excitation light which is adjustable to produce light of different wavelengths and the control means is adapted to adjust the source to produce light having the required wavelength for each exposure.

138. Apparatus as claimed in claim 136 or 137 wherein the control means is adapted in use to effect the wavelength change between the end of one exposure and the beginning of the next.

139. Apparatus as claimed in any of claims 123 to 138 wherein the control means is adapted in use to control the linear drive mans so that linear axis motion is alternatively in one direction and then in another

140. Apparatus as claimed in any of claims 97 to 139 wherein the specimen is carried by a microscope stage and means is provided to drive the stage along X and Y axes to

bring into view other portions of a sample such as for example other cells on the same slide or in a multiwell plate, or other regions of an area of tissue, or of a single sample.

141. Apparatus as claimed in any of claims 97 to 140 wherein the excitation light source produces light of more than one wavelength at the same time.

142. Apparatus as claimed in claim 141 wherein the intensity of the light at the different wavelengths differs but peaks in intensity at one or a number of the different wavelengths.

143. Apparatus as claimed in any of claims 97 to 142 wherein the excitation light source comprises one or more laser light sources.

144. Apparatus as claimed in any of claims 97 to 142 wherein a single excitation light source is employed, the wavelength or wavelengths of the light emitted therefrom can be altered, and the control means is adapted in use to adjust the source to produce light of a desired wavelength or wavelengths.

145. Apparatus as claimed in claim 144 wherein the light source is a laser light source which comprises an acousto-optical tuneable filter (AOTF) crystal, and the control means is adapted to provide in use signals to alter the frequency controlling signal to the crystal, to control the wavelength (or wavelengths) of the emitted light.

146. Apparatus as claimed in claim 144 or 145 wherein in use the control means is adapted to alter the wavelength or wavelengths only between image capture device exposures or excitations.

147. Apparatus as claimed in any of claims 97 to 142 wherein the excitation light source is a luminescent or incandescent light source and an optical filter or a monochromator

is provided which in use controls the wavelength or wavelengths of the light emitted by the source.

148. Apparatus as claimed in any of claims 97 to 142 wherein the excitation light source is a directly driven laser diode.

149. Apparatus as claimed in any of claims 97 to 147 wherein in use the excitation light source is operated so as to produce pulses of light.

150. Apparatus as claimed in any of claims 97 to 149 wherein in use the excitation light source produces polarised light.

151. Apparatus as claimed in any of claims 97 to 146 wherein the excitation light source is a light emitting diode (LED) source.

152. Apparatus as claimed in any of claims 97 to 151 wherein in use the control means is adapted to adjust the intensity of the excitation illumination incident on the specimen.

153. Apparatus as claimed in claim 152 further comprising a plurality of neutral density filters and means under the control of the control means, which in use selectively positions one or more of the neutral density filters in the light path, to achieve the intensity variation.

154. Apparatus as claimed in claim 152 further comprising an adjustable iris diaphragm in the light path and drive means operated by the control means, to open or close the iris as required.

155. Apparatus as claimed in claim 152 wherein in use the control means controls the excitation light intensity by adjusting the power to the excitation light source.

156. Apparatus as claimed in claim 152 wherein the excitation light intensity is controlled by means of an attenuating element and the control means is adapted in use to control or position the attenuating element as appropriate.

157. Apparatus as claimed in claim 156 wherein the attenuating element is an AOTF or LCD shutter.

158. Apparatus as claimed in any of claims 152 to 157 wherein the control means is adapted in use to alter the intensity of the illumination so as to provide a predetermined intensity of illumination at the specimen for each wavelength, to remove variation in intensity from one wavelength to another as can occur due to inherent intensity variation as between one source and another or between different modes of operation of the excitation light source.

159. Apparatus as claimed in any of claims 97 to 158 wherein in use the control means is adapted to adjust the power to the excitation light source and/or control attenuation of light therefrom, from one exposure to another, to provide substantially constant intensity luminescence, to reduce variation in the intensity of the light incident on the image capture device sensor due to differing wavelengths of excitation light, or to render the light emitted due to luminescence of similar intensity irrespective of wavelength, or both.

160. Apparatus as claimed in any of claims 152 to 159 wherein the intensity variation is only altered between exposures.

161. Apparatus as claimed in any of claims 97 to 160 wherein the image capture device is a CCD camera which provides a picture signal in analogue or digital format or both.

162. Apparatus as claimed in claim 160 wherein means is provided to cool the CCD camera, to increase the S/N ratio of the camera output signals.

163. Apparatus as claimed in any of claims 97 to 160 wherein the image capture device is a CMOS camera, a CID (charge injection device) camera, an intensified or gated camera, a photomultiplier tube array, a photodiode array or an image capture device employing an addressable microcolometer array, or a chemical film camera, or any other light sensitive sensor.

164. Apparatus as claimed in any of claims 97 to 163 further comprising image forming means by which the light from the specimen is formed in use into an image, which can be viewed by the human eye through an eyepiece, or can be projected onto a screen.

165. Apparatus as claimed in claim 164 further comprising a beam-splitting or beam directing device in the light path to the image capture device by which light is split or divided between the image capture device and the image forming means.

166. Apparatus as claimed in any of claims 97 to 164 further comprising two or more image capture devices.

167. Apparatus as claimed in claim 166 wherein at least one of the image capture devices is different from the other device or from at least one of the other devices.

168. Apparatus as claimed in claim 166 or 167 further comprising a beam splitting means or beam directing means in the light path to the one or more image capture devices.

169. Apparatus as claimed in any of claims 97 to 168 further comprising an optical fibre bundle by which light is transmitted to the, or each, image capture device sensor.

170. Apparatus as claimed in any of claims 97 to 169 further comprising a data storage means adapted to store signals from the or each image capture device.

171. Apparatus as claimed in any of claims 97 to 170 further comprising computer means programmed to process and analyse the signals from the or each image capture device or data storage means.

172. Apparatus as claimed in any of claims 97 to 171 further comprising a display device and means for supplying signals from the or each image capture device or from data storage means for displaying an image of the signals for visual analysis.